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LUTEIN SEQUESTRATION AND FURANOCOUMARIN METABOLISM IN PARSNIP WEBWORMS UNDER DIFFERENT ULTRAVIOLET LIGHT REGIMES IN THE MONTANE WEST

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Abstract—Both biotic and abiotic selection pressures can contribute to geographic variation in allelochemical production in plants. We examined furanocoumarin production in western North American populations of *Heracleum lanatum* and *Pastinaca sativa* that, at different latitudes and altitudes, experience different ultraviolet (UV) light regimes. Total furanocoumarins and linear furanocoumarins of fruits were negatively correlated with UV irradiance, whereas amounts of angular furanocoumarins, which are generally less phototoxic, were not. Another factor potentially influencing furanocoumarin production is the presence of the parsnip webworm *Depressaria pastinacella*, (Lepidoptera: Oecophoridae), an herbivore that feeds on reproductive structures of both plant species. These insects sequester lutein from their host plants; this carotenoid acts to ameliorate furanocoumarin toxicity. Although the concentration of lutein in fruits did not vary with UV irradiance, lutein sequestration by sixth instars was positively correlated with UV irradiance. Webworm populations are variably infested with the polyembryonic webworm parasitoid *Copidosoma sosares* Walker (Hymenoptera: Encyrtidae). *H. lanatum* fruits from populations with webworms parasitized by *C. sosares* had lower concentrations of furanocoumarins, with the exception of sphondin, than fruits from plants infested with webworms free from parasitism. Lower levels of these furanocoumarins may reduce negative effects on the fitness of this parasitoid. In contrast with the variation

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in furanocoumarin content, the ability of webworms to metabolize furanocoumarins by cytochrome P450 did not differ significantly among populations from New Mexico to Alberta.

Key Words—Furanocoumarin, cytochrome P450, ultraviolet light, carotenoid, lutein, altitude, tritrophic interaction, geographic mosaic, *Depressaria pastinacella*, *Heracleum lanatum*, *Pastinaca sativa*, *Copidosoma sosares*.

INTRODUCTION

Chemical defenses in plants against biotic stress agents function in environments that influence their efficacy and, therefore, their adaptive value to the plant (Baldwin and Preston, 1999). Among plant allelochemicals that are particularly dependent on abiotic conditions are phototoxins, chemicals whose toxicity is enhanced by exposure to light (Arnason et al., 1992). Many phototoxic plants grow preferentially in sunlit habitats and maximize exposure of herbivores to photoactivating wavelengths (Berenbaum, 1981; Arnason et al., 1992). In that light itself can cause damage to plants, many phototoxic plants also produce antioxidant allelochemicals that ameliorate light-induced oxidative damage; the content of these antioxidant compounds in plants varies in response to oxidative stress created by high light exposure (Frankel and Berenbaum, 1999; Polle et al., 1999; Hansen et al., 2002). Because variation in light intensity can exist across the host range of a phototoxic plant and its herbivores, the relative benefit of a phototoxic allelochemical as a herbivore defense may vary on a landscape scale.

Specialist herbivores of phototoxic plants have behavioral and physiological adaptations that limit the effects of photoactivation (Aucoin et al., 1990, 1995; Arnason et al., 1992). Among these counteradaptations is sequestration of plant antioxidants that when ingested along with phototoxins can reduce toxicity (Ahmad and Pardini, 1990; Aucoin et al., 1995). Chief among these sequestered plant antioxidants are carotenoids and xanthophylls (Valadon and Mummery, 1978; Rothschild et al., 1986; Aucoin et al., 1990; Fields et al., 1990). These tetraterpenoids function as accessory pigments, photoprotectants, and antioxidants in photosynthetic tissues (Britton, 1993); as singlet oxygen quenchers and free radical scavengers (Burton and Ingold, 1984; Krinsky, 1989), carotenoids allow plants to cope with oxidative stress (Larson, 1988). Because light exposure presents oxidative challenges to herbivores as well as plants, carotenoids and xanthophylls also reduce phototoxicity in insects (Robertson and Beatson, 1985; Aucoin et al., 1990; Green and Berenbaum, 1994).

Lepidopterans do not synthesize carotenoids but rather acquire them directly from host tissues with little modification (Feltwell, 1978; Kayser, 1985). Among lepidopterans that consume phototoxic plants, most sequester lutein, the

predominant carotenoid in angiosperm photosynthetic tissues (Ahmad and Pardini, 1990; Britton, 1993). This selective sequestration allows lepidopteran specialists on phototoxic plants to reduce effects of phototoxins before and during photoactivation (Rothschild et al., 1986; Ahmad and Pardini, 1990).

One herbivore that encounters host plant phototoxins under a variety of light conditions is the parsnip webworm *Depressaria pastinacella*, an oligophagous caterpillar that feeds on the reproductive tissues of plants in the genera *Heracleum* and *Pastinaca* (Hodges, 1974; McKenna and Berenbaum, 2003). The interaction of the parsnip webworm with its host plants is mediated by furanocoumarins, phototoxic compounds activated by ultraviolet A (UVA) light (320–400 nm). Furanocoumarins cause damage in the presence of UVA by directly binding to DNA and proteins, and by forming reactive oxyradicals and free radicals that damage biologically sensitive molecules (Berenbaum, 1991). The parsnip webworm tolerates high concentrations of host plant furanocoumarins through rapid detoxification by cytochrome P450 monooxygenases (Nitao, 1989). In populations of the parsnip webworm and the wild parsnip *Pastinaca sativa*, its predominant host plant in the midwestern United States, furanocoumarin production by the plant and detoxification capabilities of the webworms are genetically variable traits subject to reciprocal selection pressures (Berenbaum et al., 1986; Berenbaum and Zangerl, 1992; Zangerl and Berenbaum, 1997). Parsnip webworms also cope with furanocoumarin phototoxicity by sequestering lutein and other xanthophylls from host plant tissues (Carroll et al., 1997). This selective sequestration of dietary lutein is associated with decreased behavioral avoidance of UVA light, presumably because of decreased oxidative stress from furanocoumarins (Carroll et al., 1997).

Although examined most intensively in the Midwest, the interaction between the parsnip webworm and its apiaceous host plants occurs throughout much of North America. The parsnip webworm was introduced to eastern North America about 150 yr ago and spread to disturbed habitats throughout eastern and northern North America (Riley, 1889; Berenbaum and Zangerl, 1991). It has since spread west and become established throughout the Pacific Northwest and the intermontane region of the western United States and southwestern Canada, although it occurs much more sporadically in the southern part of its range (Hodges, 1974; McKenna and Berenbaum, 2003).

Throughout much of its range, the parsnip webworm has acquired another host plant, a native cow parsnip, *Heracleum lanatum* (Berenbaum and Zangerl, 1991). In many western locales, the webworm utilizes the more abundant *H. lanatum* exclusively. Webworms feeding on *H. lanatum* fruits encounter concentrations of phototoxic furanocoumarins significantly different from webworms feeding on *P. sativa* fruits (Berenbaum and Zangerl, 1991; Zangerl and Berenbaum, 2003). Relative to eastern and midwestern congeners, western webworms encounter high intensities of photoactivating UVA light through the

southern half of their introduced range because of the occurrence of host plants at higher altitudes in lower latitudes (Young, 1985; Blumthaler et al., 1997). Through the combined effects of altitude and latitude on UVA light intensity, populations of *D. pastinacella* in western North America may vary more than twofold in experience of maximum UVA exposure along a UVA gradient from British Columbia to New Mexico.

The ability of webworms to act as selective agents on host plant chemistry may be affected not only by abiotic factors, such as UV intensity, but also by biotic factors, such as the presence of a third trophic level, e.g., a specialist parasitoid. Webworms in New Mexico, Utah, and southeastern Idaho are heavily attacked by the polyembryonic egg–larval parasitoid *Copidosoma sosares* Walker, an encyrtid wasp native to Europe that specializes on *Depressaria* spp. (Ode et al., 2004). *C. sosares* has not been previously observed in webworm populations in other regions of North America that lack webworm parasitoids of comparable impact (Ode et al., 2004). Significant rates of parasitism can lead to decreased selection pressure on host plant chemical defenses because of relaxation of herbivory (Price et al., 1980; but see Coleman et al., 1999). At the same time, host plant chemical defenses may negatively affect parasitoid fitness, especially if the parasitoid is more sensitive to the allelochemicals than its herbivore host (Reitz and Trumble, 1996; Ode et al., 2004; Ode, 2006). Dietary furanocoumarins can limit the size of polyembryonic parasitoid broods as well as the growth and development of individual parasitoid larvae in the host (Reitz and Trumble, 1996). Thus, host plants may experience selection for reduced chemical defense to increase parasitoid fitness (Turlings and Benrey, 1998; Ode et al., 2004).

Throughout its North American host range, the parsnip webworm has been introduced into a mosaic of different UVA light intensities, host plant furanocoumarin levels, and parasitoid infestation levels. In this study, we characterized the effects of geographically variable abiotic (UV) and biotic (parasitism) factors on the interaction between parsnip webworms and their apiaceous host plants. To quantify photochemical stresses encountered by webworms in these populations, we measured host plant furanocoumarin content. Because furanocoumarins are metabolically expensive to synthesize (Zangerl and Berenbaum, 1997) and because their toxicity is enhanced by photoactivating UVA, furanocoumarin content should be lower in populations that experience high UVA light intensities. We quantified individual linear and angular furanocoumarins separately in fruits because individual furanocoumarins differ in their contributions to resistance against the parsnip webworm (Berenbaum et al., 1986). Given the importance of metabolism in resistance to furanocoumarins (Nitao, 1989), we also compared the cytochrome-P450-mediated metabolism of furanocoumarins in webworms from different populations (Berenbaum et al., 1986; Berenbaum and Zangerl, 1998). Webworms experience higher mortality on host plant

TABLE 1. POPULATIONS OF *Depressaria pastinacella* COLLECTED IN WESTERN NORTH AMERICA, ARRANGED FROM NORTH TO SOUTH, WITH LATITUDE (N) AND LONGITUDE (W) LISTED BELOW EACH LOCATION

Population	Latitude/longitude	Host plant	No. of plants	Percentage attacked	Percentage parasitized
Hixon, BC (HXBC)	53°15.760/122°28.129	*H. lanatum*	52	77	0
Cypress Hills, AB (CHAB)	49°36.279/110°15.554	*H. lanatum*	132	42	0
Birch Bay, WA (BBWA)	48°87.807/122°46.232	*H. lanatum*	76	24	0
Lost Airport, WA (AIWA)	48°60.608/120°44.488	*H. lanatum*	34	100	0
Bumblebee Creek, ID (BUID)	47°69.439/116°14.182	*H. lanatum*	56	13	0
Route 152, ID (RTID)	47°48.525/115°58.799	*H. lanatum*	82	73	0
Shoshone Creek, ID (SHID)	47°45.303/115°58.706	*H. lanatum*	150+ est.	45 est.	0
Montpelier Canyon, ID (MCID)	42°19.851/111°14.003	*H. lanatum*	250+ est.	25 est.	97 (2000) 48 (2001)
Lower Paris, ID (LPID)	42°09.853/111°23.939	*P. sativa*	10,000+	40 est.	88 est. (2001)
Payson Lakes, UT (PLUT)	39°56.185/111°38.415	*H. lanatum*	1500 est.	40 est. 60 est.	60 est. (2001) 90 est. (2000)
Dalton Canyon, NM (DCNM)	35°46.519/105°41.622	*H. lanatum*	45	9	60 (2001)
Little Tesuque, NM (LTNM)	35°43.727/105°50.342	*H. lanatum*	134	69	3 (2001)
Big Tesuque, NM (BTNM)	35°39.523/105°48.641	*H. lanatum*	168	9	20 (2001)

Percent of plants attacked by *D. pastinacella* and webworms parasitized by the encyrtid *Copidosoma sosares* are given (for small populations) or estimated (for populations > 100). All the populations except for BBWA and SHID represent new locales for the parsnip webworm.

chemotypes that do not match their metabolic phenotype, presumably because of inefficient detoxification and subsequent accumulation of unmetabolized furanocoumarins (Berenbaum and Zangerl, 1998; Zangerl and Berenbaum, 2003). By examining populations with mismatched profiles, factors that vary predictably on a landscape scale can be identified that alter the efficacy of traits mediating the interaction (Thompson, 1999; Zangerl and Berenbaum, 2003).

Because UV irradiance also affects carotenoid production in plants (Jahnke, 1999), we measured lutein content of host plants across the

altitudinal/latitudinal gradient as well as lutein sequestration in sixth instar webworms from western populations experiencing different UV light regimes as a result of latitude and altitude. Arthropods can increase antioxidants and pigmentation in response to high UVA intensities (Vega and Pizarro, 2000; Borgeraas and Hessen, 2002). If parsnip webworms use dietary lutein to reduce oxidative stress associated with furanocoumarins, greater sequestration would be expected in western populations that experience high UVA radiation.

Finally, because parasitism by *C. sosares* constitutes a significant biotic factor that affects the chemical mediation of the interaction of western webworms and their host plants, we examined the effect of the presence of *C. sosares* on host plant furanocoumarin chemistry by comparing furanocoumarin content of fruits from populations with and without the parasitoid.

METHODS AND MATERIALS

Sampling of Insect and Plant Populations. We examined 13 populations of parsnip webworms and their host plants in western North America along a latitudinal and altitudinal gradient from 35.4°N to 53.2°N (Table 1; Figure 1). For one of these populations (LPID), *P. sativa* was the exclusive host, whereas *H. lanatum* was the exclusive host for the other 12 populations. In six populations in New Mexico, Utah, and southeastern Idaho, parsnip webworms were attacked by *C. sosares* (Table 1). These parasitoid populations represent a new record for *C. sosares* in North America (Ode, personal communication). Parasitism rates of webworms varied considerably among populations and years, ranging from 5% (Dalton Creek, NM) to over 90% (Payson Lakes, UT) of the sixth instars present, creating a mosaic of attacked and unattacked webworms across the North American range of this parasitoid (Table 1).

To minimize the degradation of lutein, samples for chemical analysis were collected directly onto powdered dry ice (Oliver and Palou, 2000). Half-filled fruits, which are regularly consumed by sixth instar webworms (Berenbaum et al., 1986), were sampled from host plants for analysis of furanocoumarins and lutein. Sixth instars collected for lutein quantification were separated from plant material and isolated for 2 hr to clear plant material from their guts before placement on powdered dry ice. Samples were kept in a −80°C freezer until extraction.

FIG. 1. Approximate locations of *Depressaria pastinacella* populations sampled in western North America. Populations are labeled by acronyms presented in Table 1 and the text. *Pastinaca sativa* is the only host plant present in the LPID population, whereas *Heracleum lanatum* is the only host plant found in all other populations.


HXBC
BBWA
AIWA
CHAB
BUID
RTID
SHID
MCID
LPID
PLUT
DCNM
LTNM
BTNM

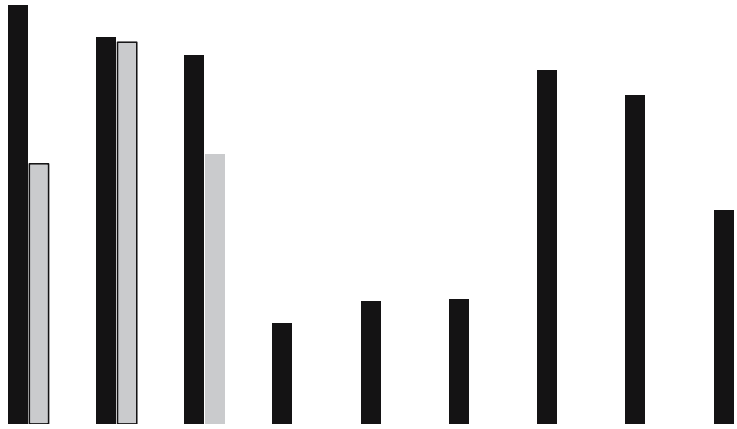
TABLE 2. ESTIMATION OF DAILY UV IRRADIANCE (DUV) FROM THE COMPARISON OF COLLECTION SITE CHARACTERISTICS (LATITUDE, ALTITUDE, COLLECTION DATE) WITH DATA FROM THE NATIONAL ULTRAVIOLET MONITORING CENTER (NUVMC) MONITORING STATION

Population	Latitude (N)	Altitude (m)	Date	Station	Station latitude	$Elevation (m)	DUV (J/cm^2)
HXBC	53°15.760	702	08/09/01	Olympic	48°51	196	4596
CHAB	49°36.279	1243	08/08/01	Glacier	48°51	267	4272
BBWA	48°87.807	2	07/02/00	Olympic	48°51	−6	4797
AIWA	48°60.608	805	07/02/00	Olympic	48°51	799	5218
SHID	47°45.303	1439	07/03/00	Glacier	48°51	463	6198
MCID	42°19.851	1911	07/18/00	RMNP	40°03	−985	6427
LPID	42°09.853	1829	07/18/00	RMNP	40°03	−1067	6363
PLUT	39°56.185	2404	07/14/01	Canyonlands	38°47	1106	8516
DCNM	35°46.519	2250	07/13/01	Albuquerque	35°09	635	8340
LTNM	35°43.727	2560	07/13/01	Albuquerque	35°09	945	8603
BTNM	35°39.523	3006	07/13/01	Albuquerque	35°09	1391	8983

The latitude and collection date from the population site are used to determine on which day the monitoring station has the same solar angle as the population site. The raw DUV value acquired for this day is corrected for the difference in elevation between the population and monitoring station sites by methods described in the text. The acronyms for population sites are described in Table 1. NUVMC monitoring stations: Glacier—Glacier National Park; Olympic—Olympic National Park; RMNP—Rocky Mountain National Park; Canyonlands—Canyonlands National Park; Albuquerque—Albuquerque).

Estimates of Daily UV Irradiance. To estimate daily UV exposure at a population site, we used measurements of total daily UV irradiance collected by the National Ultraviolet Monitoring Center (NUMC; University of Georgia and Environmental Protection Agency, 2003) at seven sites throughout North America (Table 2). At each site, the total daily UV irradiance was measured by a Brewer spectrophotometer as the total combined flux of solar UVA (320–360

FIG. 2. Tissue concentrations of (A) total furanocoumarins, linear furanocoumarins, and angular furanocoumarins; (B) the linear furanocoumarins bergapten and imperatorin; (C) the linear furanocoumarins isopimpinellin and xanthotoxin; and (D) the angular furanocoumarins angelicin and sphondin in half-filled fruits of *H. lanatum* and *P. sativa* from populations in western North America. Populations are labeled by acronyms presented in Table 1 and the text. *P. sativa* is the only host plant present in the LPID population, whereas *H. lanatum* is the only host plant found in all other populations. Content means for each class of furanocoumarin significantly differ across populations by one-way analysis of variance (ANOVA; $P < 0.05$). Populations that do not share a superscript letter have significantly different mean contents by Tukey's honestly significantly different (HSD) test ($P < 0.05$). Error bars indicate one standard error ($N = 18$–20 for each population, 176 total).



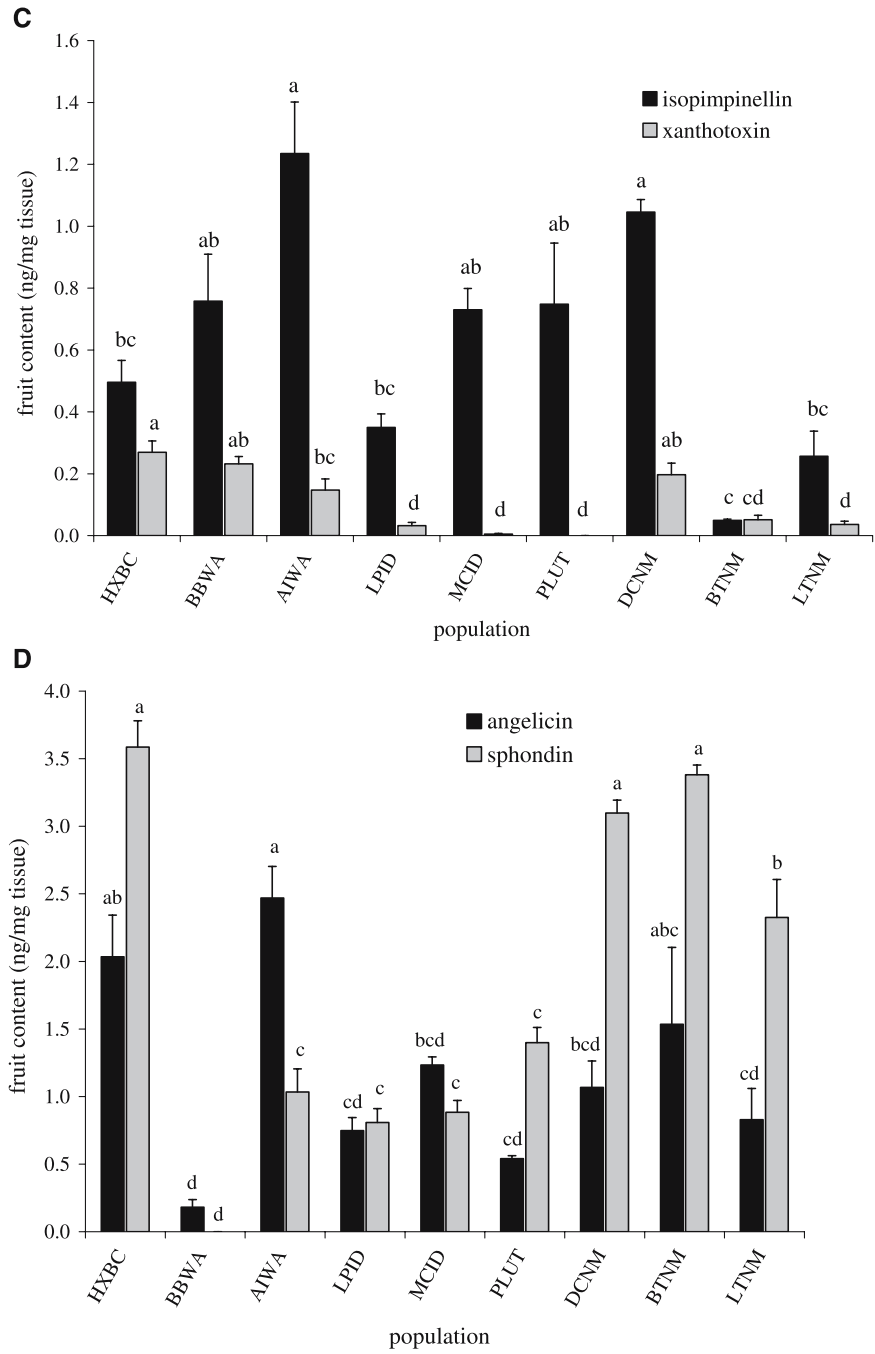


FIG. 2. (continued).

nm) and UVB irradiance (290–320 nm). To calculate daily UV irradiance by using the NUMC data, we had to compensate for differences in latitude and altitude between the sampling site and the monitoring station. The intensity of solar radiation varies with latitude on a given day, primarily because of differences in the angle of incidence between the sun and the horizon, as measured by the maximum solar angle at solar noon. However, sites at different latitudes may receive the same amount of UV irradiance on different days if they share the same maximum solar angle. To correct for differences in angle of incidence due to latitude, the maximum solar angle at solar noon was determined for the sampling site for the day of collection by using the site's latitude and longitude with a solar position calculator (NOAA, 2003). We then used the coordinates of the UV monitoring station and the solar position calculator to determine the precise day on which the UV monitoring station experienced the same maximum solar angle as the sampling site. Because UV irradiance is positively correlated with altitude due to a reduction in the amount of radiation intercepted or scattered by air molecules (Diffey, 1991; Schmucki and Philipona, 2002), the daily UV irradiance obtained from the NUMC data was corrected for differences in altitude as:

$$(\text{daily UV irradiance}) \times (1+(\Delta\ \text{altitude})(\text{altitude effect}))$$

where $ altitude is [(altitude (sampling site) − altitude (UV monitoring site)] and the altitude effect, or change in the intensity of radiation with altitude, is 0.000107 per meter based on the studies conducted during the summer with recording stations over a wide range of elevation in midlatitude mountains (Blumthaler et al., 1997; Schmucki and Philipona, 2002).

Furanocoumarin Analysis of Plant Samples. H. lanatum and *P. sativa* furanocoumarins were quantified as in Berenbaum and Zangerl (1992). Freeze-dried fruits were weighed, crushed in an Eppendorf tube with a micropestle, and extracted with 1 ml ethyl acetate. Normal-phase liquid chromatography (HPLC) was performed on a Waters high-pressure liquid chromatograph with a Model 440 absorbance (254 nm) detector. Ten microliters of each diluted sample were auto-injected and separated on an Alltech Absorbosphere Silica 5U (150 × 4.6 mm) with a 55:32:3 cyclohexane/isopropyl ether/butanol eluent mixture. Furanocoumarins were quantified by comparison of the integrated peaks against a furanocoumarin standard containing known quantities of bergapten (Sigma Inc., St. Louis, MO, USA), xanthotoxin (Sigma Inc., St. Louis, MO, USA), imperatorin (Serva, Heidelberg, Germany), isopimpinellin (Indofine, Belle Mead, NJ, USA), angelicin (Sigma Inc., St. Louis, MO, USA), and sphondin (gift of Dr. W. Wulff, University of Chicago). Sample size (number of plants) varied from 18 to 20, depending on the number of intact half-filled fruits available from each population.

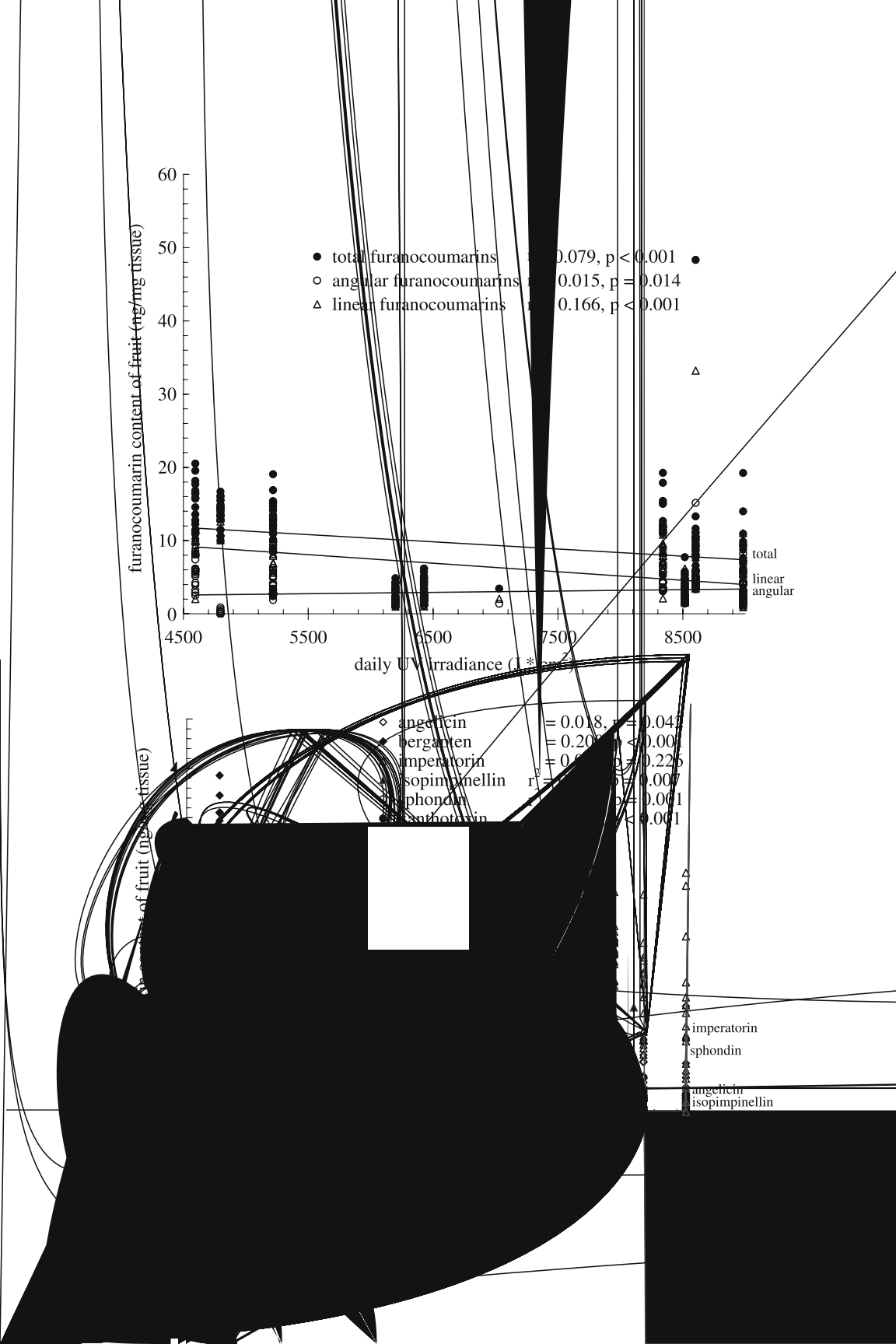

furanocoumarin content of fruit (ng/mg tissue)
daily UV irradiance (J * m²)
total furanocoumarins
angular furanocoumarins
linear furanocoumarins
total
linear
angular
angelicin
bergapten
imperatorin
isopimpinellin
sphondin
xanthotoxin
imperatorin
sphondin
angelicin
isopimpinellin

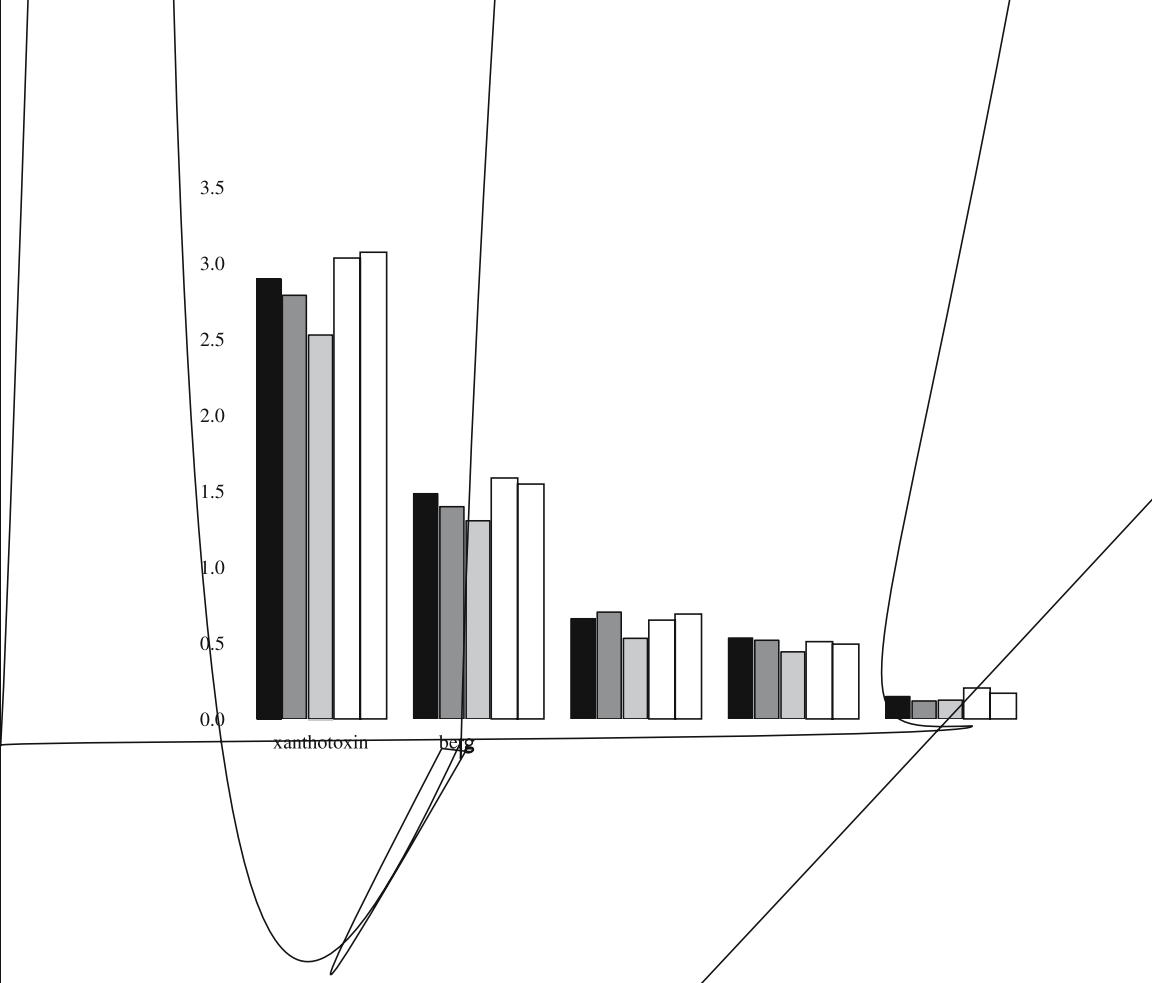
TABLE 3. DIFFERENCES IN FURANOCOUMARIN CONTENT OF *Heracleum lanatum* FRUITS FROM POPULATIONS WHERE THE WEBWORM PARASITOID *C. sosares* WAS PRESENT AND FRUITS FROM POPULATIONS WHERE THIS PARASITOID WAS ABSENT

	Concentration (2g/mg dry mass)				
	Population				
Furanocoumarin	*C. sosares* absent	*C. sosares* present	*t* value	*df*	*P* value
Angelicin	1.546 (0.182)	1.043 (0.133)	2.235	114.906	0.027
Bergapten	4.243 (0.605)	0.670 (0.027)	5.898	57.223	<0.001
Imperatorin	5.549 (0.417)	3.531 (0.395)	3.512	139.221	0.001
Isopimpinellin	0.841 (0.089)	0.564 (0.629)	2.548	111.873	0.012
Sphondin	1.469 (0.213)	2.208 (0.117)	−3.032	91.751	0.003
Xanthotoxin	0.214 (0.020)	0.057 (0.011)	7.024	90.846	<0.001
Linear furanocoumarins	10.848 (0.382)	4.822 (0.411)	10.745	148.520	<0.001
Angular furanocoumarins	3.015 (0.335)	3.250 (0.192)	−0.608	94.684	0.544
Total furanocoumarins	13.863 (0.353)	8.072 (0.577)	8.563	148.024	<0.001

Furanocoumarin means are compared by an independent samples *t* test. Standard error is given in parentheses. Degrees of freedom (*df*) are corrected in each test because equal variances of samples are not assumed. Sample size is 58 from populations where *C. sosares* is present and 98 from populations where *C. sosares* is absent.

Furanocoumarin Metabolism in Parsnip Webworms from Different Populations. Because dietary exposure to plant components affects larval P450 enzyme activity, field-caught webworms were reared to adulthood and mated to obtain offspring reared under common environmental conditions. Parsnip webworms collected as late instar larvae or pupae were allowed to emerge as adults, then subjected to photoperiod and temperature parameters simulating winter conditions to break reproductive diapause (Nitao and Berenbaum, 1988). "Overwintered'' adults were caged with wild parsnip leaves at 20°C and 16:8 light/dark photoperiod to allow for mating and oviposition. Neonates were reared to sixth instar on a semidefined artificial diet that lacks a significant carotenoid or furanocoumarin source (Carroll et al., 1997). Five of

FIG. 3. Two-dimensional plot of furanocoumarin concentrations of *H. lanatum* half-filled fruits against estimated daily ultraviolet (UV) irradiance for (A) linear, angular, and total furanocoumarins as well as (B) the individual furanocoumarins angelicin, bergapten, imperatorin, isopimpinellin, sphondin, and xanthotoxin. Univariate regression analysis was conducted for combined linear (bergapten, imperatorin, isopimpinellin, and xanthotoxin), combined angular (angelicin and sphondin), and total furanocoumarins (all six furanocoumarins) as well as the individual compounds. For each compound, a best-fit regression line has been added. The r^2 value and *P* value from the linear regression model are presented above each regression line ($N = 176$).



the 13 populations (Little Tesuque, NM; Big Tesuque, NM; Dalton Creek Canyon, NM; Lower Paris/Bear Lake, ID; Cypress Hills, Alberta) from which webworms were collected produced sufficient number of larvae to be used in metabolism bioassays. Sample size varied from 17 to 49, depending on the availability of sixth instars reared from parents collected in each population.

Midgut metabolism rates for five furanocoumarins (xanthotoxin, bergapten, isopimpinellin, imperatorin, and sphondin) in sixth instars were compared among populations according to Berenbaum and Zangerl (1992). Whole midguts were dissected, rinsed, and homogenized with a Model 985-370 Tissue Tearor (Biospec Products, Bartlesville, OK, USA). A portion of the homogenate (70 2l) was added to 1 ml phosphate reaction buffer (pH 7.8) containing a mixture of xanthotoxin, bergapten, isopimpinellin, imperatorin, and sphondin. The reactions were run for 15 min in a 30°C water bath and terminated by exposure to an 85°C water bath for 5 min. Unmetabolized furanocoumarins were extracted

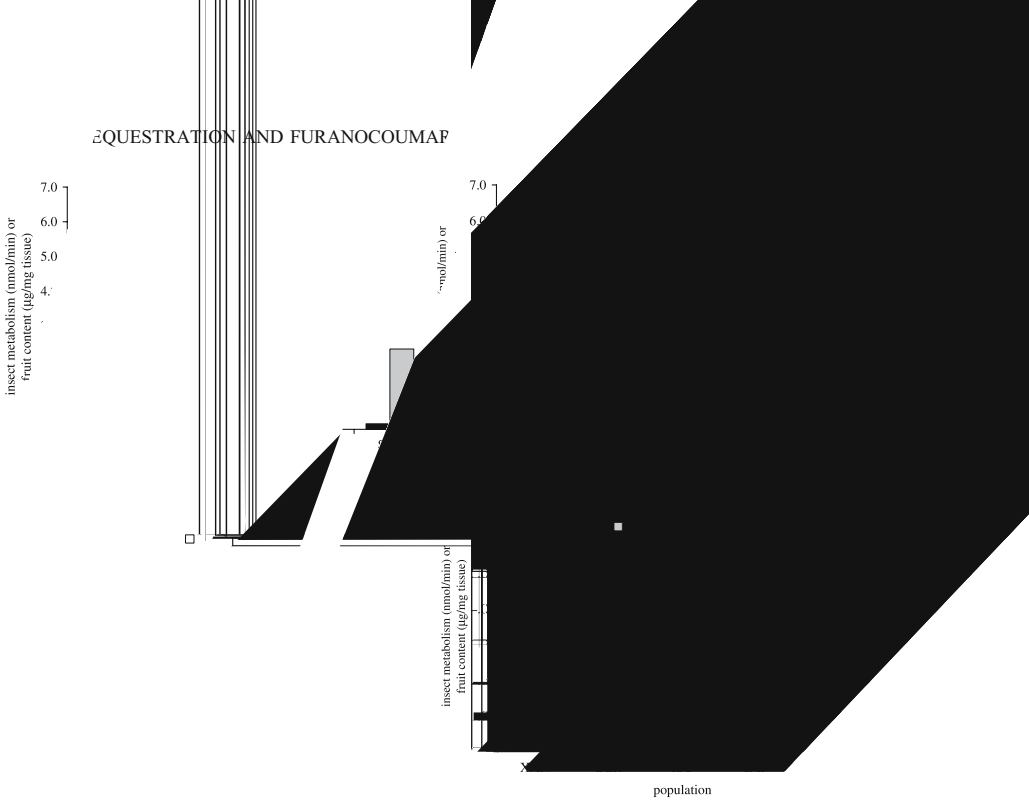
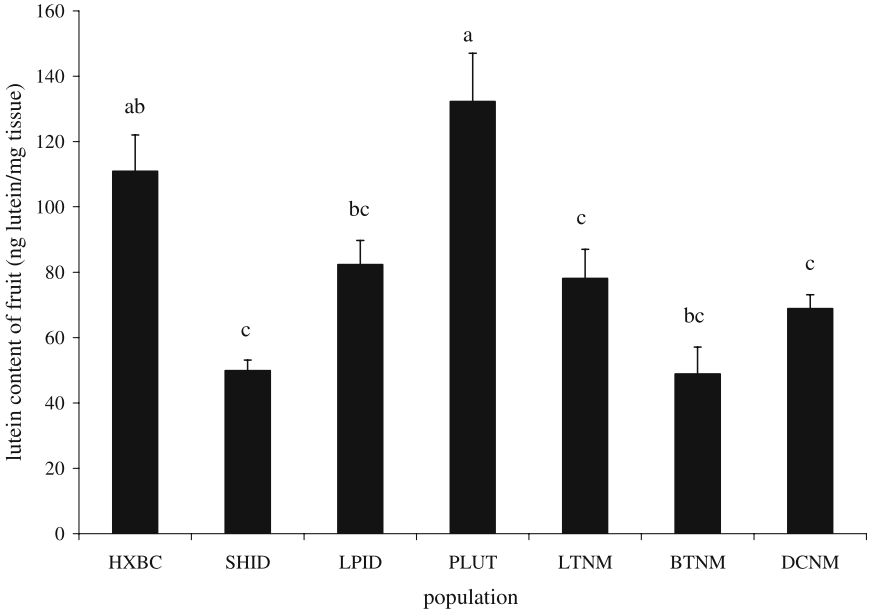


FIG. 5. Host plant f anocoumarin concentrations of half-filled fruits and furanocoumarin m tabolism rates of sixth instar *D. pastinacella* f DCNM, LTNM, nd LPID populations in western North Americ units for metabo ism apply to the webworms, whereas the units f the host plants. *P. sativa* is the only host plant present in the LP *H. lanatum* is the only host plant in all . Fu half-filled f uits and webworm ates opulatio (LTNM—I M— ig Tesuq fID)

with e l acetate and analyzed by mal-phase HPLC (Berenbaum and Zange.i, 1992). The amount of e furanocoumarin metabolized in each reac ion was determined by com on against time-zero controls.

Lutein Analysis of Plan ples. To quantify lutein in parsnips from (ierent populations, plan rial was extracted according to a protocol odified after Riso and . (1997) and O'Neil and Schwartz (1992). All amples were kept f n dry ice and extracted under minimal light onditions to reduc tion of lutein because of oxygen, light, and heat. n plant ma weighed and crushed to a powder in liquid nitrogen Because of the sensitivity of plant material to lutein not dried to obtain a direct measure of sample dry dered plant sample was weighed,


lutein content of fruit (ng lutein/mg tissue)
160
140
120
100
80
60
40
20
0
ab
c
bc
a
c
bc
c
HXBC
SHID
LPID
PLUT
LTNM
BTNM
DCNM
population

removed, and freeze-dried for later calculation of a wet mass–dry mass conversion factor. Approximately 200 mg of powdered material were placed in a preweighed Eppendorf tube, weighed, and extracted with 1 ml methanol containing 0.1% of the antioxidant butylhydroxytoluene (BHT) (Oliver and Palou, 2000). The samples were vortexed and sonicated in a Model 250 Ultrasonic Cleaner (RAI Research) for 2 min and then placed on ice for 30 min under a nitrogen atmosphere to maximize the extraction of lutein while minimizing oxidation. The sample mixture was then microcentrifuged for 5 min at 14,000 rpm at 5°C. Samples were stored in the dark at −80°C until separation by reverse-phase HPLC. Sample size varied from 19 to 39 for each population.

Reverse-phase HPLC was performed with a Waters 996 photodiode array (PDA) detector with detection of xanthophylls at 445 nm. Separation of lutein from other xanthophylls was improved by the use of a Vydac 201TP54 C_{18} column (Yeum et al., 1996). Twenty microliters of each sample were auto-injected and separated with a 90:10 methanol/acetonitrile eluent mixture containing 0.01% BHT and 0.05% of the solvent modifier triethylamine (Oliver and Palou, 2000). Three-dimensional UV–VIS spectral data were captured, analyzed, and quantified with Millenium^{32} System software. Lutein was identified and quantified by comparison of an integrated peak against a purified lutein standard generously supplied by Kemin, Inc. (Des Moines, IA, USA).

Lutein Analysis of Insect Samples. To extract lutein from webworms, preweighed whole frozen webworms were crushed to a powder in liquid nitrogen in a 2.0-ml Eppendorf tube with a micropestle. The powdered remains were extracted with 1 ml methanol containing 0.1% BHT, vortexed, sonicated for 2 min, and placed on ice for 30 min under a nitrogen atmosphere. The sample mixture was then microcentrifuged for 8 min at 14,000 rpm at 5°C to separate solid remains and refractory lipids from the lutein extract. Lutein was isolated by HPLC as described. Sample size varied from 11 to 14, depending on the availability of sixth instars from each population.

Statistical Analysis. For the half-filled fruits, amounts of total furanocoumarin, total linear furanocoumarin, and total angular furanocoumarin, as well as individual furanocoumarins and lutein concentrations, were compared among

FIG. 6. Lutein content of (A) half-filled fruits of *P. sativa* (LPID) and *H. lanatum* (all other populations) and (B) sixth instar *D. pastinacella* from populations in western North America. Populations are labeled by acronyms presented in Table 1 and are arranged left to right from north to south. *P. sativa* is the only host plant present in the LPID population, whereas *H. lanatum* is the only host plant in all other populations. Lutein content differs significantly across populations by a one-way ANOVA ($P < 0.05$). Populations that do not share a superscript letter have significantly different mean contents by Tukey's HSD test ($P < 0.05$). Sample size for each population varied from 19 to 39 for fruits and 11 to 14. Error bars indicate one standard error.


A
9000

populations by one-way analysis of variance (ANOVA). Insect lutein and whole midgut metabolic rates (for each furanocoumarin) were also compared among populations by one-way ANOVA. Treatment means were compared among individual populations by Tukey's honestly significantly different test (SPSS, 1999). Univariate regression analysis was conducted to determine the effect of daily UV irradiance on plant and insect chemistry (SPSS, 1999).

To determine the effect of the presence of the parasitoid *C. sosares* on host plant chemistry, we compared the furanocoumarin concentration of cow parsnips from populations where the parasitoid was present with cow parsnips from populations where the parasitoid was absent by independent sample *t* tests (SPSS, 1999). Separate tests were conducted for total furanocoumarins, linear furanocoumarins, and angular furanocoumarins, as well as each individual furanocoumarin.

To evaluate the match between host plant furanocoumarin production and webworm metabolic capabilities within a population, we examined the interaction between furanocoumarins (host plant furanocoumarins and midgut metabolism capability) and organism (wild parsnip or parsnip webworm) as compared by repeated-measures multivariate analysis of variance (MANOVA), with furanocoumarins as the between-subject factor and organism as the within-subject factor (SPSS, 1999). Mean levels of furanocoumarin production by plants and midgut metabolism by webworms were compared separately for each population.

RESULTS

Furanocoumarin Analysis of Plant Samples. Significant differences were observed among populations for all measures of half-filled fruit furanocoumarin concentration (Figure 2, one-way ANOVA). Both total furanocoumarins and total linear furanocoumarins of fruits were negatively correlated with daily UV irradiance ($r^2 = 0.079$ and 0.166, respectively, $P < 0.001$; regression analysis), whereas total angular furanocoumarin content of fruits was not correlated with UV light exposure ($r^2 = 0.015$, $P = 0.054$; regression analysis; Figure 3). Among the individual furanocoumarins in fruits, angelicin ($r^2 = 0.018$, $P = 0.042$), bergapten ($r^2 = 0.200$, $P < 0.001$), isopimpinellin ($r^2 = 0.035$, $P = 0.007$), and

FIG. 7. Two-dimensional plot of lutein concentrations of (A) *H. lanatum* half-filled fruits and (B) sixth instar *D. pastinacella* against estimated daily UV irradiance for the host plant's population. A regression line has been added, with the r^2 value and *P* value from the linear regression model presented above the line. Linear regression models indicate that daily UV irradiance is a good predictor of webworm, but not fruit, lutein content ($N = 155$ for fruits and 75 for webworms).

xanthotoxin ($r^2 = 0.168$, $P < 0.001$) were negatively correlated with daily UV irradiance, whereas sphondin ($r^2 = 0.130$, $P < 0.001$) was positively correlated with daily UV irradiance (Figure 3). Imperatorin ($r^2 = 0.003$, $P = 0.226$) was not significantly correlated with daily UV irradiance (Figure 3).

The furanocoumarin concentration of fruits from populations with and without *C. sosares* differed significantly for all individual furanocoumarins tested (Table 3). Fruits from populations with *C. sosares* present had lower concentrations of total furanocoumarins and linear furanocoumarins than fruits from populations where *C. sosares* was absent ($P < 0.05$, independent samples t test; Table 3). Total angular furanocoumarins did not differ in the presence or absence of *C. sosares* ($P > 0.05$, independent samples t test; Table 3). Among the individual furanocoumarins, angelicin, bergapten, imperatorin, isopimpinellin, and xanthotoxin were lower and sphondin was higher in *H. lanatum* populations where *C. sosares* was present ($P < 0.05$, independent samples t test; Table 3).

Furanocoumarin Metabolism in Parsnip Webworms from Different Populations. Despite the disparate geographical origins of the five webworm populations, the midgut metabolic rates for all five furanocoumarins did not differ among sixth instars ($P < 0.05$; one-way ANOVA; Figure 4). For four of these populations, fruit concentrations and insect metabolism rates were compared for five individual furanocoumarins. In all four, a significant interaction between furanocoumarin and organism type occurred in a comparison of fruit concentration and midgut metabolism [Pillai's Trace = 0.901 (BTNM), 0.915 (DCNM), 0.936 (LPID), and 0.958 (LTNM); $P < 0.001$; MANOVA], indicating that the relative amount of individual furanocoumarins metabolized by insects differed from the plant furanocoumarin profile (Figure 5). In particular, webworm capacity for metabolizing xanthotoxin proportionally exceeded the relatively low production of xanthotoxin in fruits, whereas webworm capacity for metabolizing imperatorin and sphondin was disproportionately less than the relative amounts of these two furanocoumarins in fruits.

Lutein Analysis of Plant and Insect Samples. Although mean lutein concentration of fruits varied among populations ($P < 0.05$, one-way ANOVA; Figure 6), daily UV irradiance was not a good predictor of fruit lutein ($r^2 = 0.001$, $P = 0.282$; regression analysis; Figure 7). By contrast, webworms from populations exposed to more intense daily UV irradiance had higher lutein ($r^2 = 0.236$, $P < 0.001$; regression analysis; Figure 7), with significant differences observed across the populations ($P < 0.05$, one-way ANOVA; Figure 6).

DISCUSSION

At high altitudes, *H. lanatum* produces less total furanocoumarin and linear furanocoumarins but not angular furanocoumarins in half-filled fruits than

plants under less intense UV light regimes at lower altitudes. *H. lanatum* from populations in the central and southwestern United States (New Mexico, Utah, and southern Idaho) produced more sphondin (range 0.9–3.5 2g/mg) and less xanthotoxin (range 0.0–0.2 2g/mg) than cow parsnips from the Midwest (0.4 2g/mg sphondin and 1.1 2g/mg xanthotoxin; Zangerl and Berenbaum, 2003). A similar pattern pertains to *P. sativa*: western wild parsnip populations from Lower Paris, ID, have higher fruit content of sphondin (0.9 2g/mg) and lower content of bergapten (0.6 2g/mg) and xanthotoxin (0.03 2g/mg) than parsnips from the Midwest (0.3 2g/mg sphondin, 1.9 2g/mg bergapten, and 4.0 2g/mg xanthotoxin; Zangerl and Berenbaum, 2003) or the Netherlands (0.5 2g/mg sphondin, 2.2 2g/mg bergapten, and 2.7 2g/mg xanthotoxin; Ode et al., 2004).

The differences between linear and angular furanocoumarins under high UV intensities may be partially explained by the relative importance of photoactivation to overall toxicity. Because linear furanocoumarins form diadducts with pyrimidine bases in the presence of photoactivating UVA light, their phototoxicity is greatly enhanced relative to their dark toxicity (Berenbaum, 1991). Most of the toxic effects of linear furanocoumarins against insect herbivores are attributed to photogenotoxicity rather than photooxidation (Berenbaum, 1991). In contrast, angular furanocoumarins form only monoadducts with pyrimidine bases because of steric inhibition, resulting in a more modest increase in phototoxicity over their dark toxicity and a greater reliance on photooxidation overall. In addition, the efficacy of furanocoumarin photogenotoxicity increases under lower oxygen partial pressures (Bianchi et al., 1996), such as those experienced at high altitudes. Because enhancement of linear furanocoumarin toxicity by UVA is proportionally greater and more dependent on photogenotoxicity, increases in UVA intensity at high altitudes should improve the efficacy of linear furanocoumarins more than angular furanocoumarins. Under consistently greater UVA light intensities experienced, proportionately fewer linear furanocoumarins yield equivalent toxicity toward herbivores. In *P. sativa*, furanocoumarin biosynthesis has a significant cost in terms of reproductive fitness (Zangerl and Berenbaum, 1997). If similar costs occur in *H. lanatum*, a reduction in linear furanocoumarin production could allow plants to shift resources to more costly production of angular furanocoumarins (Berenbaum et al., 1986; Zangerl and Berenbaum, 1997), resulting in the increase in sphondin observed here.

Decreased production of phototoxic allelochemicals in plants under high light intensities has also been observed with benzylisoquinoline alkaloids, compounds activated in the near UV spectrum. Larson et al. (1991) compared the production of phototoxic isoquinoline alkaloids in the alpine columbine *Aquilegia caerulea* and the low-elevation columbine *A. canadensis* under ambient and elevated UVB radiation. Alkaloid concentrations in *A. caerulea* under elevated UVB were significantly lower than *A. canadensis* or *A. caerulea*

under ambient UVB, an effect the authors attributed to increased UVB-mediated destruction of the alkaloids; the possibility that reduced production may represent an adaptation to increased efficacy of chemical defenses under higher light intensities was not considered. Although isoquinoline alkaloids are photoactivated by near UV rather than UVB radiation (Larson et al., 1991), increases in near UV and UVB occur concomitantly under natural light conditions.

Previous studies of allelochemical variation along altitudinal and latitudinal transects have found a general trend toward lower concentrations of allelochemicals at high altitudes and latitudes, which has usually been attributed to reduced herbivory (Salmore and Hunter, 2001, but see Koptur, 1985 and Preszler and Boecklen, 1996). It is unlikely that differences in insect herbivory account for the results obtained in our study; in our limited survey over 2 yr, rates of attack by *D. pastinacella* in infested populations of *H. lanatum* did not vary consistently with UV intensity, altitude, or latitude. However, differences in furanocoumarin concentrations across populations may have arisen in response to selection pressures from other herbivores, including deer, tule elk, moose, black bear, and grizzly bear, as well as domesticated cattle (McMillan, 1953; Hamer et al., 1991; Holcroft and Herrero, 1991; Ralph and Pfister, 1992; Gogan and Barrett, 1995; Ramcharita, 2000).

An alternative and not mutually exclusive explanation for the differences in host plant furanocoumarins is the impact of the parasitoid *C. sosares*, which occurred in populations that experienced high ambient UV intensity at high altitudes. Compared to populations from western North America that lack a major parasitoid, plant populations associated with webworms parasitized by *C. sosares* display furanocoumarin profiles consistent with increased fitness of the parasitoid (Ode et al., 2004). In the Netherlands, the likelihood of parasitoid attack was negatively correlated with host plant isopimpinellin content, and the survivorship of all male and mixed-sex broods was negatively correlated with xanthotoxin content. Parsnips from the Netherlands had lower content of all linear furanocoumarins, including isopimpinellin and xanthotoxin, relative to parsnips from the midwestern United States, where *C. sosares* is absent; lower furanocoumarin content may be a response to selection for greater parasitoid fitness. In our survey of western *H. lanatum*, concentrations of all furanocoumarins except sphondin were lower in fruits from populations where *C. sosares* was present than in fruits from populations attacked by webworms in the absence of parasitoids. The furanocoumarin chemistry of host plants from these populations appears more conducive to the survival of the parasitoid and may have facilitated its ability to adapt to the parsnip webworm's host plant switch to the novel North American host plant *H. lanatum*.

The impact of *C. sosares* on host plant chemistry due to increased webworm mortality may be limited, because the host completes larval feeding before it is killed by the parasitoid (Ode et al., 2004); rather, *C. sosares* could

indirectly exert strong selection pressure on the host plant through changes in host physiology and behavior (Turlings and Benrey, 1998; Coleman et al., 1999). Koinobiont parasitoids can alter the foraging behavior (Karban and Englishloeb, 1997), developmental time (Harvey et al., 1999), consumption rates (Rahman, 1970; Slansky, 1978; Coleman et al., 1999), larval performance (Rahman, 1970; Coleman et al., 1999), and metabolism (Rahbé et al., 2002) of their hosts, all of which can be components of herbivore selection pressures on host plant chemistry.

That furanocoumarin metabolism rates did not vary among webworms from different populations in western North America contrasts with previous findings (Berenbaum and Zangerl, 1998; Zangerl and Berenbaum, 2003) that metabolic rates of midwestern webworms on wild parsnip differ across distances as small as 1 km. In these midwestern populations, phenotype mismatching increased with the presence of an alternative host plant (Zangerl and Berenbaum, 2003). We found significant overall differences between metabolic capabilities and host plant furanocoumarins in four western webworm populations despite the fact that only one host species was present. If anything, western webworm metabolic profiles resemble the furanocoumarin profiles of wild parsnip plants from the Midwest more closely than the host plants actually utilized by these caterpillars (Berenbaum and Zangerl, 1998; Zangerl and Berenbaum, 2003). These western webworms display metabolism rates near the maximum rates reported for webworms in the Midwest (Table 4; Berenbaum and Zangerl, 1998; Zangerl and Berenbaum, 2003); these rates may represent the maximum given the genetic variation present in North America.

If limits on P450 metabolism as a means of resistance against furanocoumarins exist, webworms from the southwestern populations may have other mechanisms that reduce the efficacy of host plant furanocoumarin defenses. The patterns of lutein sequestration among populations of *D. pastinacella* from western North America are consistent with the idea that webworms offset the stress of furanocoumarin toxicity by greater incorporation of dietary lutein under high UVA intensities, despite an absence of differences in host plant lutein concentrations. By sequestering carotenoid pigments from their host plants, parsnip webworms may extend the amount of time they remain exposed to UV light. Although exposure to photoactivating light can damage a specialist herbivore on phototoxic plants, complete light avoidance during foraging has costs in terms of reduced internal temperatures and increased developmental time (Rawlins and Lederhouse, 1981; Ali et al., 1990). In addition, increased pigmentation in itself can significantly improve absorption of solar radiation and raise internal body temperatures during foraging, resulting in faster development (Price et al., 1980; Goulson, 1994).

Increased pigmentation is a common characteristic in montane populations, where stress from high UV intensities, suboptimal foraging temperatures, and

TABLE 4. COMPARISON OF MIDGUT METABOLISM RATES OF FURANOCOUMARINS BY SIXTH INSTARS FROM POPULATIONS IN THE WESTERN AND MIDWESTERN UNITED STATES

Host plants present	Furanocoumarin metabolism rate (nmol/min)				
	Carroll and Berenbaum (2006)		Zangerl and Berenbaum (2003)		Berenbaum and Zangerl (1998)
Geographic location (Population number)	*P. sativa*	*H. lanatum*	*P. sativa*	*P. sativa* and *H. lanatum*	*P. sativa*
	ID (1)	ID, NM, UT (3)	IL, WI (16)	WI (4)	IL, MN (4)
Furanocoumarin					
Bergapten	1.40	1.31–1.58	0.75–1.07	0.93–1.29	0.31–0.96
Imperatorin	0.52	0.44–0.53	0.27–0.43	0.20–0.44	n/a
Isopimpinellin	0.70	0.53–0.68	0.31–0.49	0.47–0.72	0.05–0.13
Sphondin	0.12	0.12–0.20	0.07–0.12	0.02–0.19	0.12–0.17
Xanthotoxin	2.79	2.53–3.07	1.28–2.43	1.68–2.13	0.57–1.72

The average furanocoumarin metabolism rates for each study are reported along with the geographic location (by state) and number of the populations covered by the study. If metabolism rates were quantified for more than one population in the study, a range of population means for furanocoumarin metabolism rates is presented. Because the presence of alternative host plants has a significant effect on the average metabolism rates in a population, metabolism rates are subdivided into categories by the webworm host plants (*H. lanatum* or *Pastinaca sativa*) present in or near the source populations.

the risk of oxidative damage from freezing are generally higher than at lower elevations (Bidigare et al., 1993; Jung et al., 1998). Conversely, pigmentation can increase convective cooling losses in microhabitats that experience wind and reduced irradiation, thereby reducing the chance of overheating in direct sunlight (Willemsen and Hailey, 1999). In summer, the montane habitat of the parsnip webworm in the Southwest is dominated by the North American monsoon, which results in frequent formation of cloud cover and thunderstorms in foothills and mountains (Adams and Comrie, 1997). Increased pigmentation by lutein sequestration may allow for thermal heating during sunny intervals while reducing stress related to furanocoumarin phototoxicity.

The higher rates of lutein sequestration observed in the montane populations may also be a result of the enhancement of carotenoid antioxidant and photoprotectant activities in the low oxygen partial pressures (pO_2) experienced at these altitudes. The ability of carotenoids to ameliorate both photogenotoxicity and photooxidation mechanisms is dependent on the ambient pO_2, with loss of function occurring in heavily oxygenated tissues (Burton and Ingold, 1984; Palozza et al., 1997; Bianchi et al., 1996; Eichler et al., 2002). Because of the high efficiency of the insect tracheal respiratory system, where pO_2 declines

only slightly from the external atmosphere to the smaller tracheoles that penetrate tissues (Tenney, 1985; Timmins et al., 1999), the efficacy of lutein as an antioxidant in webworm tissues is largely dependent on the pO_2 of the surrounding atmosphere. At the altitudes of the New Mexico and Utah populations (above 2200 m), the partial pressure of oxygen is at least 25% lower than at sea level. The pO_2 of anoxic internal cavities that encounter high concentrations of furanocoumarins, such as the midgut lumen (Johnson and Barbehenn,

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